IS 326-19 (1998): Method of Sampling and Test for Natural and Synthetic Perfumery Materials, Part 19: GAS Chromatograpfilc Analysis of Perfumery Materials [PCD 18: Natural and Synthetic Fragrance Materials]

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भारतीय मानक

प्राकृतिक और संश्लेषित सुगन्ध सामग्री — नमूने लेने और परीक्षण की पद्धतियाँ

भाग 19 सुगंधित सामग्री का गैस वर्णलेखी विश्लेषण

Indian Standard

METHOD OF SAMPLING AND TEST FOR NATURAL AND SYNTHETIC PERFUMERY MATERIALS

PART 19 GAS CHROMATOGRAPHIC ANALYSIS OF PERFUMERY MATERIALS

ICS 71:100.70

BUREAU OF INDIAN STANDARDS
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March 1998

Price Group 2

FOREWORD

This Indian Standard (Part 19) was adopted by the Bureau of Indian Standards, after the draft finalized by the Natural and Synthetic Perfumery Materials Sectional Committee had been approved by the Petroleum, Coal and Related Products Division Council.

Gas Liquid Chromatography is a fast, simple, accurate and ideal technique for analysis. It is of special value for both qualitative and quantitative analysis of mixtures containing organic compounds. It is gaining far greater importance in the analysis of natural and synthetic perfumery materials and essential oils which are complex mixtures of volatile terpenic compounds.

GLC is a form of chromatography which accomplishes the separation of a vapourizable sample by partitioning the sample between a mobile gas phase and a stationary liquid phase usually coated on a solid support.

In practice GLC is applicable to any liquid organic substances that are easily vapourized. Solids that have high vapour pressures at room temperature, are dissolved in a volatile solvent for GC analysis. In some instances, non-volatile substances may be converted to derivatives which are vapourizable under the conditions that obtain in the chromatographic process. Time for running the GC on packed column is to be given as 45 min and for capillary column as 60 min.

It should be noted that this GLC specification is for the procedural guidelines and information purposes only. To get complete details of the operation of this technique, on the job training is recommended.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHOD OF SAMPLING AND TEST FOR NATURAL AND SYNTHETIC PERFUMERY MATERIALS

PART 19 GAS CHROMATOGRAPHIC ANALYSIS OF PERFUMERY MATERIALS

1 SCOPE

This standard (Part 19) prescribes the gas chromatographic techniques for the analysis of pure natural and synthetic perfumery materials and essential oils.

2 OUTLINE OF THE METHOD

A small amount of the perfumery material is introduced into a gas liquid partition column. The various components that are volatile under the conditions of test are vapourized and transported through the column by a carrier gas. The separated components are measured in the effluent by a detector and recorded as a chromatogram. The chromatogram is interpreted by applying component attenuation and detector response factors to the peak areas, and the relative concentrations are determined by relating the individual peak responses to the total peak responses.

3 APPARATUS

3.1 The system which enables GLC analysis, is a standard gas chromatograph which consists of:

a) Oven
b) Injection Port
c) Columns
d) Detector
e) Temperature Controllers
f) Carrier Gas and Flow Regulators
g) Recorder and Data Processor
h) Power Supply

A block diagram of a typical gas chromatograph is shown in Fig. 1.

3.1.1 *Oven*

An oven is the important part of a gas chromatograph, well equipped with an efficient heavy duty heater, a fan to provide uniform temperature distribution inside the oven chamber, Swage lock fittings for Injection Port and Detectors for fixing columns; Temperature Sensor and Controller. The Oven should be well insulated to maintain temperature from ambient to 300°C with a variation of only ± 0.5°C. The oven is also equipped with temperature programmer's facility wherein the desired temperatures can be raised gradually at prefixed rates. Modern chromatographs have microprocessor computerised controls for heating oven, injection port, detector and temperature programming as per pre-recorded programmes entered in the microprocessor.

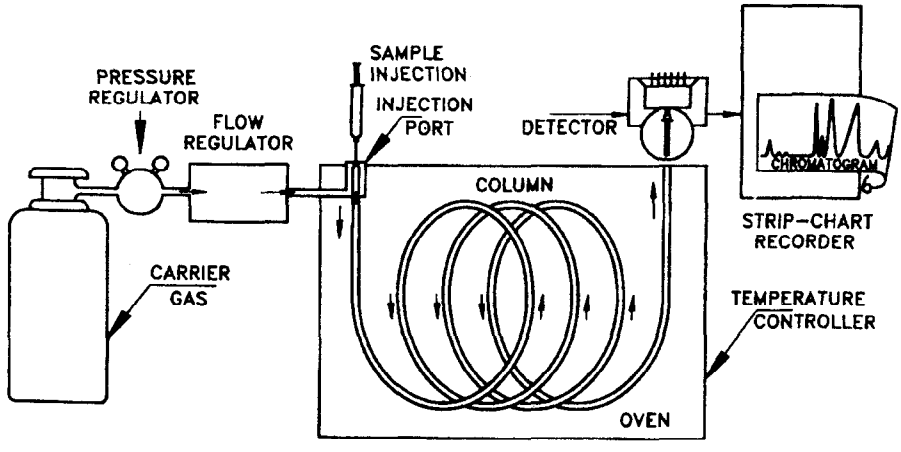

FIG. 1 A TYPICAL GAS CHROMATOGRAPH

3.1.2 *Injection Port/Injection Block*

The injection port is an extension of the chromatographic column inlet, accommodating the sample that is injected with a microlitre hypodermic syringe through a self sealing silicone rubber septum which seals the injection port from outside. The temperature of the injection block is controlled by a temperature controller and is usually adjusted so as to be approximately 25-50°C higher than the highest boiling fractions in the sample. The high temperature ensure flash evaporation of the sample and the flowing carrier gas sweeps the vapourized sample on to the stationary phase of the chromatographic column. The volume of the injection block is kept to a minimum to avoid unnecessary dilution of the sample with the carrier gas, resulting in chromatographic peak broadening.

3.1.2.1 *Sample injection*

Sample injection into a gas chromatograph requires different methods which depend upon the phase of the sample and the type of the column employed (Open Tubular, Capillary or Packed). Nevertheless, requirements are the same for all injection methods:

a) High degree of reproducibility and precision, and
b) Injection of the sample in the shortest possible time and smallest possible volume.

Practical sample sizes for the different columns are:

a) 0.1 to 0.2 microlitre of the material in a 30 to 50 percent solution of pure analytical grade acetone or cyclohexane for capillary column, and
b) 0.2 to 1.0 microlitre for packed columns.

With capillary columns, special type of split/splitless and capillary inlets are used, where split ratio of usually 50:1 or 100:1 can be adjusted with the aid of a needle valve. This enables only 1/50 or 1/100 of the sample quantity injected, actually reaching capillary column and the rest is allowed to vent out. This avoids unnecessary over loading of the capillary column.

3.1.3 *Column*

The column is the most important part of GLC as it is responsible for separation. A column is a long, coiled tube made of stainless steel, copper, glass of fused silica either filled permeably with a granular substance, a well dispersed liquid phase on a solid, inert support (packed column) or coated on the inside with a high boiling liquid phase (capillary or open tubular column or support coated columns) causing sample components to separate. The choice of a suitable standard columns depends upon the level of resolution required. The packing material of the column could be polar or non-polar depending on the specific application as recommended in the literature.

3.1.4 *Detector*

It is that part of the gas chromatograph which employs some physical or chemical properties of the eluted sample components or column effluents to indicate their presence in the carrier gas. Various techniques are used to detect the chemical components as they emerge from the column. A suitable type of detector can be used for specific application. The types of commonly used detectors are:

a) Thermal conductivity,
b) Flame ionisation detector,
c) Electron capture detector, and
d) Mass spectroscopic detector.

3.1.5 *Temperature Controllers*

The rate at which an organic substance moves through and is ultimately off the GLC column depends upon temperature. Actually there are three temperatures to consider:

a) *Injection port* — It is essential that all of the sample gets vapourized instantaneously when it is injected into the injection port. The injection port must therefore be heated to a temperature at which the component with highest boiling point will vaporize.
b) *Column* — The column temperature must be uniform whether it is in isothermal mode or programming.
c) *Detector* — The detector temperature is usually set, so that effluent from the column does not collect on the detector. A temperature about 20°C higher than that of injection port is usually sufficient. The temperature of injection port, column and detector must remain constant throughout the chromatographic operation. If the chromatograph is to be heated up or if the temperature settling is to be changed, enough time must be allowed for thermal equilibrium to be established.

3.1.6 *Carrier Gas*

Although carrier gases are available in substantially pure form from steel cylinders where they are stored under pressure, yet only certified grade gases should be used with gas chromatographs having packed or capillary columns. This would ensure long life for the columns. The carrier gas is pre-dried to make completely moisture free by passing through traps filled with silica gel and molecular sieves.

The choice of a carrier gas depends upon the type of detector being employed. Hydrogen and Helium, for example, are particularly suited for use with a thermal conductivity type of detector because they both

possess high thermal conductivity, and hence allow a rapid response by the detector. With flame ionization detector only exceptionally pure nitrogen is used.

3.1.6.1 *Flow controllers*

The rate of flow of the carrier gas through columns is to be of highest order of precision. This is very essential to ensure proper elution of components in the chromatogram and to have a steady baseline throughout in both isothermal and temperature programming modes of operation. Flow controllers in GLC take care of this requirement.

3.1.7 *Power Supply*

The gas chromatograph system must be attached to an electro mechanical mains stabilizer to ensure that the mains power supply does not fluctuate more than ± 1 percent of the specified voltage (220/230 Volts AC, 50 cycles). A constant voltage transformer (C.V.T.) should also be used with computing integrator. This is to ensure noisefree baseline during data processing of GLC analysis.

3.1.8 *Recorder and Data Processing*

The information from gas chromatographic separation is in the form of chromatograms. There is no chemical identification in most cases. The substance is identified by the time elapsed between the introduction of the sample and the appearance of its peak maximum, this time is referred to as retention time on the chromatogram. Under constant GLC conditions for a particular column, the retention time is a reproducible and characteristics feature.

There are several quantitative techniques in use for peak area measurements in a chromatogram and determining the composition of a compound. For achieving the highest accuracy in quantitative estimation of GLC analysis; electronic digital integrators would be preferred which give a complete print out of areas of all components in a chromatogram alongwith retention times. In modern integrators even datas can be stored which can be reproduced whenever desired. In analysis with capillary columns use of a electronical computing integrators is extremely essential.

4 PROCEDURE

The gas chromatograph is prepared for perfumery chemicals or essential oils under the conditions indicated in the relevant standard of the chemical oil being tested.

After stabilization of the desired temperature of the column, injection port and detector, a suitable amount of the sample is injected with microlitre hypodermic syringe. The amount of liquid samples would be 0.2 to 1.0 microlitre for packed columns and 0.1 to 0.2 microlitre of the material in a 30 to 50 percent solution of pure analytical grade acetone or cyclohexane for capillary columns. The solid sample would also be dissolved in acetone or cyclohexane and the solution would be injected in appropriate amount.

On the chromatogram obtained, all peaks of interest should be of suitable dimensions and that except in case of attenuation, none should exceed 90 percent of all the available recorder paper width. If required, sample is re-injected to get a better chromatogram.

5 CALCULATION

Peak areas are calculated either by the most commonly used triangular method or automated integration. The sum of the areas is equated to 100 percent, it is tactly implied that all substances have come out of the column and all substances have the same response factor, however, this is not the case in truth due to response factor of detector. Yet this method of calculation is fairly accurate.

6 INTERPRETATION OF CHROMATOGRAM

In most cases the interpretation of the chromatogram and of the numerical integration values associated therewith can be performed by comparison with a specific set of standards for every product to be analysed.

7 REPORT

The report of a gas chromatographic investigation should specify:

a)	The type of apparatus	
b)	Column	Material; length in m; internal diameter in mm.
c)	Column packing	Stationery phase and its weight percentage, support material and its particle size. If column packings not containing a separate stationery phase have been used this column packing should be indicated by suitable characteristics.
d)	Column temperature (°C)	Isothermal or programming. If a temperature programme has been used, the temperature limits and the rate of change in temperature in °C/min.
e)	Carrier gas and flow rate	

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Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Handbook' and 'Standards Monthly Additions'.

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Amendments Issued Since Publication

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